

Antigenic peptides and their use

FIELD OF THE INVENTION

5 This invention relates generally to the field of pathogen peptidic antigens and their use, for example, for the preparation of a vaccine against said pathogen. More specifically, the present invention relates to an antigenic peptide deriving from plasmodium species sequences, and includes antibodies and methods of producing and using same.

BACKGROUND OF THE INVENTION

10 Human Plasmodium falciparum (*Pf*) is a public health problem. Today approximately 40% of the world's population mostly those living in the poorest countries are at risk of malaria. Malaria causes more than 300 million acute illnesses and at least one million deaths
15 annually. 90% of deaths due to malaria occur in sub-Saharan African countries mostly among young children and pregnant women (<http://rbm.who.int>).

Unfortunately, vaccine discovery is still a very empirical process given that protective antigens do not bear any structural, physicochemical or sequence related characteristics that would allow their identification. The only constraint for antigens whose protective capacity is
20 antibody mediated is their accessibility to antibody molecules. Initially, vaccine development was performed with intact microorganisms or proteins, which were easily obtainable through classical purification methods. Introduction of molecular biology techniques generally improved the antigen isolation step, but difficulty in producing highly purified recombinant proteins in milligram amounts still remains; this renders this approach very costly and not
25 adaptable to high throughput screening methods. Furthermore, proper protein folding still represents an additional problem in numerous cases. Thus, overcoming the bottlenecks of manufacturing and identification of proteins as targets of protection represents a grand challenge to the scientific community.

In the past 20 years huge efforts have been made to develop a malaria vaccine with
30 over 40 phase I and II trials being conducted (Ballou *et al*, 2004, Van de Perre and Dedet, 2004). Currently, there are approximately 70 different vaccine candidates under development with at least 10 in clinical trials. The antigens used are derived from various stages of the parasite life cycle (Genton and Corradin, 2002, Ballouet *al*, 2004), with various immunisation

regimens (Dunachie and Hill, 2003) and combined with different adjuvants formulated in various delivery systems (Genton *et al*, 2002; Taylor-Robinson, 2003; Ballou *et al*, 2004).

Results obtained in clinical trials conducted in endemic situations were encouraging and showed some levels of protection (Bojang *et al*, 1998, Bojang *et al*, 2001, Gentonet *et al*, 2002) while other trials showed no protection (Nosten *et al*, 1996, Valero *et al*, 1996, Urdaneta *et al*, 1998, Graves and Gelband, 2004). To date, the most advanced malaria vaccine is RTS,S/AS02A. This vaccine comprises a large portion of the immunologically dominant NANP repeats of the circumsporozoite protein and a portion of the C-terminal region containing T cell epitopes, which is fused to hepatitis B surface antigen (Gordon *et al*, 1995), co-expressed in *Saccharomyces cerevisiae* and formulated with the strong adjuvant AS02A (Stoute *et al*, 1997). RTS,S/AS02A targets the pre-erythrocytic liver stage and protects against both clinical malaria and parasitaemia in naive (Stoute *et al*, 1997) and malaria exposed adult males (Bojang *et al*, 2001), although for a short period. In addition, better protection level against clinical malaria has been obtained in toddlers in a recently completed trial in Mozambique (Alonso *et al*, 2004). Protection was sustained for up to 6 months in children, which are the most affected by the disease.

The vaccine candidates that have been used to date have not been systematically selected for their ability to protect; most of the parasite molecules were empirically prioritised following their chronological order of discovery. This approach though successful for other pathogens, has not been able to provide an effective malaria vaccine.

For example, EP 0315 0185 B1 (in the name of Behringwerke aktiengesellschaft) described the isolation of a gene which codes for a histidine/alanine-rich protein by screening two different *Plasmodium falciparum* cDNA gene banks with antiserum against a 41kDa protein and by cross-hybridisation with the insert DNA of a clone obtained therewith. The isolated protein protected Aotus monkeys from infection with *Plasmodium falciparum*. Unfortunately, this approach had not lead to the development of an efficient vaccine in humans probably due to the fact that these monkeys are not the appropriate models to mimic human immunological response to *Plasmodium falciparum*.

In an attempt to develop vaccines against another pathogen, *Streptococcus pneumoniae*, mapping studies using monoclonal antibodies raised against pneumococcal surface protein A (PspA) indicated that the major cross-reactive epitopes are found in the last 100 amino-acids of the coiled-coil domain which is an important structural and biologically abundant domain found in diverse group of proteins (Chen and Parry, 1986 and 1990).

Pursuant to this finding, International Patent Application WO00/37105 (in the name of Medimmune, Inc.) disclosed a process and a vaccine composition for preventing infection caused by *Streptococcus pneumoniae* that comprises polypeptides and fragments of polypeptides containing histidine triad residues or coiled-coil regions.

More recently, International Patent Application WO01/96368 (in the name of Cytovax Biotechnologies, Inc.) disclosed the use of a coiled-coil structural scaffold to generate structure-specific peptides, including synthetic peptides. These synthetic peptides are useful as vaccines or to stimulate antibody production or cell-mediated immunity to the naturally occurring pneumococcal surfaces proteins A and C.

Although there has been some progress in the treatment of malaria, the development of a safe and effective malaria vaccine remains an urgent unmet medical need for vast populations living in malaria-endemic region.

This object has been achieved by providing a new antigenic peptide deriving from *Plasmodium* species comprising at least one coiled coil region. This peptide has been prepared according to a method of producing an antigenic peptide for the preparation of a vaccine against a pathogen based on a new approach for identifying the presence of a sequence encoding at least one coiled-coil region in the genome of said pathogen.

SUMMARY OF THE INVENTION

These and other objects as will be apparent from the foregoing have been achieved by providing a new antigenic peptide deriving from Plasmodium species comprising at least one
5 coiled coil region, characterized in that said antigenic peptide is selected from the group comprising the amino acid sequences SEQ ID N°1 to 132, SEQ ID N°134 to SEQ ID N°153 and SEQ ID N°159 to SEQ ID N°163, biologically active fragments thereof, molecular chimeras thereof, combinations thereof and/or variants thereof.

Another object of the invention is to provide an antigenic cocktail composition
10 deriving from Plasmodium species.

This invention also contemplates the use of the antigenic peptide deriving from Plasmodium species or of the antigenic cocktail composition in the preparation of a vaccine composition useful to stimulate an immune response in a mammal and the use thereof in the manufacture of a medicament for the treatment and/or prevention of malaria

15 A further object of the present invention is to provide an antibody that recognizes the antigenic peptide of or the antigenic cocktail composition deriving from Plasmodium species.

The present invention also relates to a purified and isolated nucleic acid sequence comprising a nucleotidic sequence encoding the antigenic peptide deriving from Plasmodium species, an expression vector comprising at least one copy of said purified and isolated nucleic
20 acid sequence, and a host cell comprising either the purified and isolated nucleic acid sequence or the expression vector.

The present invention further relates to a method of producing an antigenic peptide for the preparation of a vaccine composition against a pathogen.

A diagnostic tool for determining the presence of the antigenic peptide or antibodies
25 directed against said antigenic peptide is also contemplated in the present invention

Other objects and advantages will become apparent to those skilled in the art from a review of the ensuing detailed description, which proceeds with reference to the following illustrative drawings, and the attendant claims.

BRIEF DESCRIPTION OF THE FIGURES

5 **Figure 1** represents the immunofluorence data obtained on infected human erythrocytes using affinity purified human antibodies against SEQ ID N° 34 (P1577), SEQ ID N° 4 (P1574), SEQ ID N° 133 (MR198) and SEQ ID N°1 (MR194) .

DETAILED DESCRIPTION OF THE INVENTION

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The present invention relates to an antigenic peptide deriving from Plasmodium species comprising at least one coiled coil region from a Plasmodium species sequence.

15 The term “comprising” is generally used in the sense of including, that is to say permitting the presence of one or more features or components.

20 As used herein, the terms “protein”, “polypeptide”, “polypeptidic”, “peptide” and “peptidic” are used interchangeably herein to designate a series of amino acid residues connected to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues.

25 The term “antigenic peptide” refers to a peptide that is recognized by an antibody. Usually, the immune response results in the production of antibodies recognizing the antigenic peptide or at least a part thereof.

In the present context, the term “deriving” refers to the fact that the antigenic peptide has been selected among the amino acid sequences present in the Plasmodium species pathogen sequence.

30 “Coiled coil region”, “coiled-coil domain” or “region” are usually made up of amino acid sequence having heptad (*abcdefg*) repeats with apolar residues at the a and b positions and typically polar residues elsewhere. The structure of these regions are stabilized by

hydrophobic interactions between the α and d positions in the different strands (Hodges et al, 1981; Hodges, 1996).

“*Plasmodium* species” as used herein can be selected among the following
5 *plasmodium* strains:

Plasmodium agamae, *Plasmodium atheruri*, *Plasmodium azurophilum*, *Plasmodium berghei*,
Plasmodium brasilianum, *Plasmodium cathemerium*, *Plasmodium chabaudi*, *Plasmodium*
chiricahuae, *Plasmodium coatneyi*, *Plasmodium cuculus*, *Plasmodium cynomolgi*,
10 *Plasmodium elongatum*, *Plasmodium fairchildi*, *Plasmodium falciparum*, (such as
Plasmodium falciparum (isolate 311), *Plasmodium falciparum* (isolate 7G8) ,*Plasmodium*
falciparum (isolate CAMP / Malaysia), *Plasmodium falciparum* (isolate CDC / Honduras),
Plasmodium falciparum (isolate DD2), *Plasmodium falciparum* (isolate FC27 / Papua New
Guinea), *Plasmodium falciparum* (isolate FcB1 / Columbia), *Plasmodium falciparum* (isolate
15 FCBR / Columbia), *Plasmodium falciparum* (isolate FCH-5), *Plasmodium falciparum* (isolate
FCM17 / Senegal), *Plasmodium falciparum* (isolate FCR-3 / Gambia), *Plasmodium*
falciparum (isolate fid3 / india), *Plasmodium falciparum* (isolate hb3), *Plasmodium*
falciparum (isolate IMR143), *Plasmodium falciparum* (isolate K1 / Thailand), *Plasmodium*
falciparum (isolate KF1916), *Plasmodium falciparum* (isolate LE5), *Plasmodium falciparum*
20 (isolate mad20 / papua new guinea), *Plasmodium falciparum* (isolate mad71 / papua new
guinea), *Plasmodium falciparum* (isolate NF54), *Plasmodium falciparum* (isolate NF7 /
Ghana), *Plasmodium falciparum* (isolate nig32 / nigeria), *Plasmodium falciparum* (isolate
PALO ALTO / UGANDA), *Plasmodium falciparum* (isolate RO-33 / Ghana), *Plasmodium*
falciparum (isolate T4 / Thailand), *Plasmodium falciparum* (isolate TAK 9), *Plasmodium*
25 *falciparum* (isolate thtn / thailand), *Plasmodium falciparum* (isolate V1), *Plasmodium*
falciparum (isolate WELLCOME), *Plasmodium falciparum* 3D7), *Plasmodium fieldi*,
Plasmodium floridense, *Plasmodium fragile* ,*Plasmodium gallinaceum*, *Plasmodium*
giganteum, *Plasmodium gonderi*, *Plasmodium guanggong*, *Plasmodium heteronucleare*,
Plasmodium hylobati, *Plasmodium inui*, *Plasmodium juxtannucleare*, *Plasmodium knowlesi*
30 ,*Plasmodium lophurae*, *Plasmodium malariae*, *Plasmodium cf. malariae*, *Plasmodium*
mexicanum, *Plasmodium nucleophilum*, *Plasmodium ovale*, (malaria parasite *P. ovale*),
Plasmodium reichenowi, *Plasmodium relictum*, *Plasmodium rouxi*, *Plasmodium simiovale*,
Plasmodium simium, *Plasmodium vinckei*, *Plasmodium vivax* (such as *Plasmodium vivax*

(strain Belem) *Plasmodium vivax* (strain Salvador I)), *Plasmodium yoelii*, *Plasmodium* sp. unclassified such as *Plasmodium* sp. 909413, *Plasmodium* sp. 909414, *Plasmodium* sp. 909415, *Plasmodium* sp. 909416, *Plasmodium* sp. AP61, *Plasmodium* sp. AP62, *Plasmodium* sp. AP63, *Plasmodium* sp. AP64, *Plasmodium* sp. AP65, *Plasmodium* sp. AP66, *Plasmodium* sp. AP67, *Plasmodium* sp. AP68, *Plasmodium* sp. AP69, *Plasmodium* sp. AP70, *Plasmodium* sp. AP71, *Plasmodium* sp. AP72, *Plasmodium* sp. AP73, *Plasmodium* sp. AP74, *Plasmodium* sp. AP75, *Plasmodium* sp. AP76, *Plasmodium* sp. AP77, *Plasmodium* sp. AP78, *Plasmodium* sp. C1, *Plasmodium* sp. C2, *Plasmodium* sp. C3, *Plasmodium* sp. COLL1, *Plasmodium* sp. D1, *Plasmodium* sp. DAJ-2004, *Plasmodium* sp. E1, *Plasmodium* sp. E2, *Plasmodium* sp. ex *Aplonis atrifusca*, *Plasmodium* sp. ex *Aplonis tabuensis*, *Plasmodium* sp. ex *Foulehaio carunculata*, *Plasmodium* sp. ex *Halcyon chloris*, *Plasmodium* sp. ex *Myzomela cardinalis*, *Plasmodium* sp. ex *Ptilinopus porphyraceus*, *Plasmodium* sp. F1, *Plasmodium* sp. G1, *Plasmodium* sp. GRW11, *Plasmodium* sp. GRW2, *Plasmodium* sp. GRW4, *Plasmodium* sp. H1, *Plasmodium* sp. ORW1, *Plasmodium* sp. PA, *Plasmodium* sp. PARUS2, *Plasmodium* sp. PB, *Plasmodium* sp. pBT6, *Plasmodium* sp. pBT7, *Plasmodium* sp. pBT8, *Plasmodium* sp. pBT9, *Plasmodium* sp. PC, *Plasmodium* sp. pGRW9, *Plasmodium* sp. PH, *Plasmodium* sp. PI, *Plasmodium* sp. PU1, *Plasmodium* sp. SGS1, *Plasmodium* sp. strain KZ02, *Plasmodium* sp. strain LA09, *Plasmodium* sp. strain LA15, *Plasmodium* sp. strain OZ01A, *Plasmodium* sp. strain OZ04, *Plasmodium* sp. strain OZ08, *Plasmodium* sp. strain OZ14, *Plasmodium* sp. strain OZ25, *Plasmodium* sp. strain OZ35, *Plasmodium* sp. strain OZ35A, *Plasmodium* sp. strain OZ35B, *Plasmodium* sp. strain OZ36A, *Plasmodium* sp. strain OZ42, *Plasmodium* sp. strain OZ45, *Plasmodium* sp. strain OZ46, *Plasmodium* sp. strain PR01, *Plasmodium* sp. SYAT24, *Plasmodium vivax*-like sp).

Preferably, the antigenic peptide will derive from *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*.

Usually, the antigenic peptide deriving from *Plasmodium* species comprising at least one coiled coil region will be selected from the group comprising the amino acid sequences SEQ ID N°1 to 132, SEQ ID N°134 to SEQ ID N°153 and SEQ ID N°159 to SEQ ID N°163, biologically active fragments thereof, molecular chimeras thereof, combinations thereof and/or variants thereof.

Applicants have selected SEQ ID N°1 to 132, SEQ ID N°134 to SEQ ID N°153 and SEQ ID N°159 to SEQ ID N°163, represented in table 1, according to the method of producing an antigenic peptide described *infra*. All these peptidic sequences contain at least one coiled coil region.

Table 1

10	Sanger_1 3.030 303 pos. 497-559 Pfa3D7 pfal_chr1 PFA0170c Annotation Sanger (protein coding) hypothetical protein, cons VNNLDSTVNYMNSTGNNINNIVNNLDSTVNYMNSTGNNINNIVNNLDSTVNYMNSTGNNINNI (SEQ ID N°1)
	Sanger 3.250 325 pos. 437-473 Pfa3D7 pfal_chr1 PFA0635c Annotation Sanger (protein coding) hypothetical protein Location NHDTRINDYNKRLTEYNKRLTEYNKRLTEYTKRLNE (SEQ ID N°39)
15	TIGR_1 3.320 332 pos. 140-166 Pfa3D7 pfal_chr2 PFB0145c Annotation TIGR (protein coding) hypothetical protein Location TISSLSNKIVNYESKIEELEKELKEVK (SEQ ID N°2)
	TIGR_2 3.090 309 pos. 249-273 Pfa3D7 pfal_chr2 PFB0145c Annotation TIGR (protein coding) hypothetical protein Location IIDIKKHLEKLEKIEKEKEDLENL (SEQ ID N°5)
20	TIGR_3 3.640 364 pos. 575-601 Pfa3D7 pfal_chr2 PFB0145c Annotation TIGR (protein coding) hypothetical protein Location NIKTMNTQISTLKNQVHLLNEQIDKLN (SEQ ID N°3)
25	TIGR_4 2.930 293 pos. 537-561 Pfa3D7 pfal_chr2 PFB0145c Annotation TIGR (protein coding) hypothetical protein Location INNLNEKLEETNKEYTNLQNNYTNE (SEQ ID N°6)
	TIGR_6 2.710 271 pos. 597-621 Pfa3D7 pfal_chr2 PFB0145c Annotation TIGR (protein coding) hypothetical protein Location IDKLNNEKGTLSKISELNVQIMDL (SEQ ID N°7)
30	TIGR_7 3.000 300 pos. 999-1023 Pfa3D7 pfal_chr2 PFB0145c Annotation TIGR (protein coding) hypothetical protein Location LLSKDKEIEEKNNKIKELNNDIKKL (SEQ ID N°9)
	TIGR_8 2.800 280 pos. 1154-1178 Pfa3D7 pfal_chr2 PFB0145c Annotation TIGR (protein coding) hypothetical protein Location ICSLTTEVMELNNKKNELIEENKLN (SEQ ID N°11)
35	TIGR_9 2.690 269 pos. 1126-1150 Pfa3D7 pfal_chr2 PFB0145c Annotation TIGR (protein coding) hypothetical protein Location VDKIEEHILDYDEEINKSRSNLFQLKNE (SEQ ID N°12)
40	TIGR_10 4.050 405 pos. 1198-1224 Pfa3D7 pfal_chr2 PFB0145c Annotation TIGR (protein coding) hypothetical protein Location EIEKLNKQLTKCNKQIDELNEEVEKLN (SEQ ID N°4)
	TIGR_11 2.670 267 pos. 1178-1202 Pfa3D7 pfal_chr2 PFB0145c Annotation TIGR (protein coding) hypothetical protein Location LNLVDQGGKKLKKDVEKQKKEIEKL (SEQ ID N°10)
45	TIGR_12 2.600 260 pos. 1252-1276 Pfa3D7 pfal_chr2 PFB0145c Annotation TIGR (protein coding) hypothetical protein Location LDENEDNIKKMKSKIDDMEKEIKYR (SEQ ID N°13)
	TIGR 3.170 317 pos. 1603-1627 Pfa3D7 pfal_chr2 PFB0315w Annotation TIGR (protein coding) 41 kDa antigen Location=281907. MEQMDMKMEKIDVNMQMDVKMEQM (SEQ ID N°40)
50	TIGR 3.020 302 pos. 616-645 Pfa3D7 pfal_chr2 PFB0460c Annotation TIGR (protein coding) hypothetical protein Location=co NNVIRSKMYNIKKRISKINDELHELNSFFL (SEQ ID N°41)
55	TIGR_1 3.160 316 pos. 516-540 Pfa3D7 pfal_chr2 PFB0765w Annotation TIGR (protein coding) hypothetical protein Location LEEMKQKNKELINLNDISDELKNC (SEQ ID N°42)
	TIGR_2 3.470 347 pos. 605-633 Pfa3D7 pfal_chr2 PFB0765w Annotation TIGR (protein coding) hypothetical protein Location EKLNDMQKKLNDVNEKYKNIVECLNNYKT (SEQ ID N°43)
60	TIGR_3 3.370 337 pos. 1074-1098 Pfa3D7 pfal_chr2 PFB0765w Annotation TIGR (protein coding) hypothetical protein Location LKEKDKKINDLMKDMEKKKEEINKL (SEQ ID N°44)
	Sanger 3.340 334 pos. 798-837 Pfa3D7 pfal_chr3 PFC0235w Annotation Sanger (protein coding) hypothetical protein Locat NEIKELNNTLNKYKEEMNNYKEEIVINEKYKLEIEELCK (SEQ ID N°45)

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	IEIEREKNQLQEEIKKLQNEKNDL (SEQ ID N°100)
5	Stanford 3.670 367 pos. 604-633 Pfa3D7 chr12 PFL1235c Annotation Stanford (protein coding) hypothetical protein Location TYTSLKLNQINELTKKINILRGNDLKARK (SEQ ID N°101)
	Stanford 3.440 344 pos. 506-540 Pfa3D7 chr12 PFL1605w Annotation Stanford (protein coding) hypothetical protein Location KNDINVQLDDINVQLDDINVQLDDINQLDEINLN (SEQ ID N°102)
10	Stanford_1 3.380 338 pos. 190-214 Pfa3D7 chr12 PFL1930w Annotation Stanford (protein coding) hypothetical protein Location KEEINEKLQLLNNDLDKKNEELNIL (SEQ ID N°103)
	Stanford_2 3.190 319 pos. 3629-3653 Pfa3D7 chr12 PFL1930w Annotation Stanford (protein coding) hypothetical protein Location IKNLNNEINTLNDMLKDSEEEIRML (SEQ ID N°104)
15	Stanford_3 3.350 335 pos. 3963-3987 Pfa3D7 chr12 PFL1930w Annotation Stanford (protein coding) hypothetical protein Location LNHIINELEIKREEINQMNNKLNEL (SEQ ID N°105)
20	Stanford_4 3.540 354 pos. 4776-4800 Pfa3D7 chr12 PFL1930w Annotation Stanford (protein coding) hypothetical protein Location VKILKKRLNSISNDLEKRTTEEIEHL (SEQ ID N°106)
	Stanford_5 3.380 338 pos. 5456-5480 Pfa3D7 chr12 PFL1930w Annotation Stanford (protein coding) hypothetical protein Location INVLNDEITKLKNEINTYKNDLKNL (SEQ ID N°107)
25	Stanford_6 3.480 348 pos. 5613-5637 Pfa3D7 chr12 PFL1930w Annotation Stanford (protein coding) hypothetical protein Location INKLNQQINYLQDDINSKSDNIISL (SEQ ID N°108)
	Stanford 3.260 326 pos. 117-146 Pfa3D7 chr12 PFL2310w Annotation Stanford (protein coding) hypothetical protein, conserved NFFLEQMENDMSSTYDKMNRINMDLSKLR (SEQ ID N°109)
30	Stanford_1 3.180 318 pos. 515-543 Pfa3D7 chr12 PFL2520w Annotation Stanford (protein coding) reticulocyte-binding protein EKLYILEKSINKLKLNDINNKYQTIKK (SEQ ID N°110)
35	Stanford_2 3.430 343 pos. 723-747 Pfa3D7 chr12 PFL2520w Annotation Stanford (protein coding) reticulocyte-binding protein LNQVLEKYEELKKNINEYSKEENKL (SEQ ID N°111)
	Sanger 3.160 316 pos. 446-478 Pfa3D7 chr13_1 PF13_0065 Annotation Sanger (protein coding) vacuolar ATP synthase catalytic TSFSKYVRQLEQYFDNFDQDFLSLRQKISDILQ (SEQ ID N°112)
40	Sanger 3.070 307 pos. 16-45 Pfa3D7 chr13_1 PF13_0088 Annotation Sanger (protein coding) Myb1 protein Location=complement EKLVKHLDVIDKLIENIYDNNINLNEYINK (SEQ ID N°113)
	Sanger 3.360 336 pos. 956-980 Pfa3D7 chr13_1 PF13_0097 Annotation Sanger (protein coding) hypothetical protein Location INSHNNNNMNNNDNMNNMNNNNNNI (SEQ ID N°114)
45	Sanger_1 3.570 357 pos. 864-888 Pfa3D7 chr13_1 MAL13P1.96 Annotation Sanger (protein coding) chromosome segregation protein INEIEKKIEDIEKNINITKENLKL (SEQ ID N°115)
50	Sanger_2 3.350 335 pos. 885-909 Pfa3D7 chr13_1 MAL13P1.96 Annotation Sanger (protein coding) chromosome segregation protein LKELENKITELQSSFSYENEMKHV (SEQ ID N°116)
	Sanger 3.080 308 pos. 102-126 Pfa3D7 chr13_1 PF13_0107 Annotation Sanger (protein coding) hypothetical protein Location IDDIDRNAEQINKNYEHVKNKYEHV (SEQ ID N°117)
55	Sanger 3.210 321 pos. 14-38 Pfa3D7 chr13_1 PF13_0120 Annotation Sanger (protein coding) hypothetical protein Location IDRQRDKINELEKKLEELRSSSEEL (SEQ ID N°118)
	Sanger 3.060 306 pos. 247-270 Pfa3D7 chr13_1 MAL13P1.147 Annotation Sanger (protein coding) hypothetical protein Location NIIQIKNDIEQCQKSIKKIEDNLNTYE (SEQ ID N°32)
60	Sanger 3.260 326 pos. 2069-2105 Pfa3D7 chr13_1 MAL13P1.176 Annotation Sanger (protein coding) Plasmodium falciparum reticulocyte TIVQNSYNSFSIDINKNINDIDKEMKTLIPMLDELLNE (SEQ ID N°119)
65	Sanger_1 3.200 320 pos. 912-936 Pfa3D7 chr13_1 PF13_0198 Annotation Sanger (protein coding) reticulocyte binding protein ISELEQEFNNNNQKLDNQLDINAM (SEQ ID N°120)
	Sanger_2 3.260 326 pos. 2139-2171 Pfa3D7 chr13_1 PF13_0198 Annotation Sanger (protein coding) reticulocyte binding protein MEIKTIVQNSYNSFSIDINKNINDIDKEMKTLI (SEQ ID N°121)
70	Sanger 3.380 338 pos. 618-642 Pfa3D7 chr13_1 MAL13P1.202 Annotation Sanger (protein coding) hypothetical protein Location MLSQNNIDKLKKSNNNLNEDLRKK (SEQ ID N°122)
	Sanger 3.130 313 pos. 1135-1159 Pfa3D7 chr13_1 PF13_0239 Annotation Sanger (protein coding) hypothetical protein Location

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	PF13_0239 GIFIYNMNLRLREILKLMTDNIDTLKDKINEIKCSYAFLK (SEQ ID N° 146)
	PFD0520c TKKLNKELSEGNKELEKLEKNIKELEETNNTLENDIKV (SEQ ID N° 147)
5	PF07_0111 NFVNNYINENILNLKSVDNYLEKINNKIKDLDDNINDR (SEQ ID N° 148)
	MAL6P1.254 PDFDAYNEKLGSIQSISIDEIKKKIDNLQKEIKVANK (SEQ ID N° 149)
10	PF11_0424 QLEEKTKQYNDLQNNMKTIKEQNEHLKKNKFQSMGK (SEQ ID N° 150)
	PFD0970c ENINNMDKINNVDQNNNMDEKINNVDK (SEQ ID N° 151)
15	PF14_0574 EKGLKSLNEIKKNYDSIIEEQKNQLENLKM (SEQ ID N° 152)
	PF13_0198 TISELEQEFNNNNQKLDNQLDINAMNINILQT (SEQ ID N° 153)
20	MAL6P1.37(PEG)PFB0145c(PEG)PF14_0089 KKRNVVEELHSLRKNNYINNEEIEEIT(PEG)TISSLSNKIVNYESKIEELEKELKEVK(PEG) NITNINKNIENIKNDMSNLNNMDSNQ (SEQ ID N° 154)
	PFA0170c(PEG)MAL6P1.37(PEG) PFB0145c(PEG)PF14_0089 VNNLDSTVNYMNSTGNNINNI(PEG)KKRNVVEELHSLRKNNYINNEEIEEIT(PEG)TISSLSNKIVNYESKIEELEKELKEVK(PEG) NITNINKNIENIKNDMSNLNNMDSNQ (SEQ ID N° 155)
25	MAL13P1.304(PEG) PF08_0048 EKLKKYNNEISSLKKELDILNEKMGKCT(PEG)GGLKNSNHNLNNIEMKYNTLNNNMNSINK (SEQ ID N° 156)
30	PFB0145c(PEG)MAL13P1.304(PEG) PF08_0048 LDENEDNIKKMKSKIDMEKEIKYR(PEG)EKLKKYNNEISSLKKELDILNEKMGKCT(PEG)GGLKNSNHNLNNIEMKYNTLNNNMN SINK (SEQ ID N° 157)
	PFD0520c(PEG)PF08_0048(PEG)MAL6P1.37 TKKLNKELSEGNKELEKLEKNIKELEETNNTLENDIKV(PEG)EKLKKYNNEISSLKKELDILNEKMGKCT(PEG)KKRNVVEELHSLRKNNYINNEEIEEIT (SEQ ID N° 158)
35	PFD0520c (AcNH)KDKMHQEMEKFKKDRKNLQLNLKNTNTRKNHEFLKNKMQLVLTMMKSTADDKRFQY (SEQ ID N° 159)
	PFD0520c EIIDKDIIYMKSRINIMRENADKNNQKYDKIVSQDKMHQEMEK (SEQ ID N° 160)
40	PFD0520c IKELEETNNTLENDIKVEMNK GNLYKSRLALLKKNKVRI SKAQEIIDKDIIYMK (SEQ ID N° 161)
	PFD0520c Fmoc-SIQRIKHLEGLTKKLNKELSEGNKELEKLEKNIKELEETNNTLENDIKVEMNKGNYKSRLALLKKNKV RISKAQEIIDKDIIYMK (SEQ ID N° 162)
45	PFD0520c MRHKISENEIINKIDSINLKEVKDASACMNNYTNFISIKLKNREGIHSIQRIKHLEGL (SEQ ID N° 163)

Among these 163 peptidic sequences, 95 coiled-coil segments were randomly selected (30-70 amino acids long with the highest coiled-coil score) and are present either in the same protein or in different ones. Most of these 95 selected antigenic peptides were recognized at various degrees (5-93%) by a panel of sera from donors living in endemic areas (Table 2).

Table 2

Antibody responses against 95 peptides synthesized using 37 adult sera from Burkina Faso, 42 adult sera from Tanzania and 39 adult sera from Colombia. Results are expressed as % (value > mean negative control + 3SD), ratio (OD exp / mean OD negative control) and not determined (nd).

SEQ ID N°	Proteins	%	Ratio (%)	Mean OD	%	Ratio (%)	Mean OD	%	Ratio (%)	Mean OD
		Burkina Faso sera			Tanzanian sera			Colombian sera		
1	PFA0170c	14	3	0.168	38	10	0.154	18	8	0.151
2	PFB0145c	27	5	0.135	100	55	0.252	28	5	0.118
3		27	5	0.146	48	7	0.109	nd	nd	nd
4		16	16	0.254	40	12	0.114	21	5	0.118
5		41	32	0.236	43	29	0.219	nd	nd	nd
6		22	16	0.201	50	12	0.107	nd	nd	nd
7		4	3	0.141	24	7	0.120	nd	nd	nd
8		70	43	0.294	36	26	0.274	15	5	0.101
9		32	8	0.194	56	40	0.258	26	10	0.132
10		35	5	0.185	10	7	0.117	nd	nd	nd
11		54	32	0.285	40	12	0.456	28	28	0.156
12		65	41	0.242	74	45	0.307	26	23	0.174
13		27	27	0.214	38	24	0.149	64	26	0.170
14	PFC0245c	41	30	0.266	40	21	0.185	44	31	0.215
15		27	11	0.146	50	10	0.167	nd	nd	nd
16		35	3	0.121	31	17	0.163	nd	nd	nd
17		16	0	0.123	33	17	0.142	nd	nd	nd
18		57	32	0.210	38	7	0.100	nd	nd	nd

19	PFD0110w	27	5	0.136	38	10	0.148	nd	nd	nd
20		59	0	0.192	55	19	0.133	nd	nd	nd
21		43	3	0.179	26	10	0.098	nd	nd	nd
22		59	19	0.181	67	10	0.146	nd	nd	nd
23		24	19	0.203	7	7	0.134	nd	nd	nd
24		32	30	0.267	14	12	0.121	nd	nd	nd
25	PFD1115c	0	0	0.153	98	12	0.134	nd	nd	nd
26	PFE0570w	0	0	0.125	43	12	0.148	31	15	0.113
27	MAL6P1.37	54	30	0.265	69	33%	0.237	18	8	0.124
28		22	3	0.102	69	10%	0.113	nd	nd	nd
29	MAL7P1.162	3	0	0.122	24	10%	0.129	nd	nd	nd
30	PF11_0213	16	5	0.126	45	7%	0.128	23	3	0.138
31	PFL1135c	0	0	0.136	33	17%	0.193	36	18	0.169
32	MAL13P1.147	0	0	0.123	19	14%	0.189	nd	nd	nd
33	PF13_0277	19	8	0.125	10	7%	0.121	nd	nd	nd
34	MAL13P1.336	11	8	0.207	14	12%	0.144	nd	nd	nd
35	PF14_0045	43	24	0.258	50	21%	0.180	nd	nd	nd
36	PF14_0093	0	0	0.138	52	10%	0.133	nd	nd	nd
37	PF14_0175	22	16	0.358	21	17%	0.129	nd	nd	nd
38	PF14_0089	41	14	0.163	17	12%	0.107	28	8	0.135
87	PF11_0210	59	11	0.118	29	0%	0.093	nd	nd	nd
45	PFC0235w	59	24	0.161	48	17%	0.149	nd	nd	nd
93	PF11_0455	65	35	0.188	40	0%	0.113	nd	nd	nd
72	PF07_0014	54	11	0.155	48	10%	0.150	nd	nd	nd
56	PFD0685c	16	5	0.096	63	35	0.224	8	3	0.096
63	MAL6P1.61	19	11	0.144	40	10	0.191	nd	nd	nd
86	PF11_0207	70	57	0.554	62	48%	0.263	59	36	0.295

119	MAL13P1.176	14	3	0.097	20	13	0.290	3	3	0.098
39	PFA0635c	3	0	0.093	28	8	0.199	21	3	0.110
88	PF11_0240	5	3	0.086	28	5	0.124	nd	nd	nd
64	MAL6P1.80	22	0	0.108	15	0	0.118	nd	nd	nd
102	PFL1605w	43	32	0.352	75	43	0.220	21	8	0.111
58	PFD0985w	41	14	0.127	55	35	0.221	49	8	0.144
99	PFL0770w	51	24	0.180	36	24	0.187	26	13	0.108
61	PFE0595w	22	3%	0.100	24%	0%	0.088	nd	nd	nd
68	MAL6P1.147	59%	43%	0.288	76%	36%	0.260	31%	18%	0.199
112	PF13_0065	11	3	0.119	35	5	0.125	nd	nd	nd
125	PF14_0013	35	3	0.145	24	12	0.165	nd	nd	nd
121	PF13_0198	27	11	0.146	12	10	0.161	nd	nd	nd
153		35	22	0.130	40	25	0.143	nd	nd	nd
127	PF14_0397	19	11	0.143	24	14	0.162	nd	nd	nd
50	PFC0810c	27	14	0.152	29	19	0.175	nd	nd	nd
71	MAL7P1.13	14	8	0.121	14	5	0.130	nd	nd	nd
95	PFL0150w	5	0	0.101	53	18	0.156	nd	nd	nd
128	PF14_0444	11	8	0.126	29	12	0.139	nd	nd	nd
69	MAL6P1.131	22	14	0.141	24	14	0.178	nd	nd	nd
41	PFB0460c	5	0	0.123	21	0	0.132	nd	nd	nd
96	PFL0250w	51	32	0.280	74	31	0.281	56	28	0.164
101	PFL1235c	19	5	0.144	12	5	0.122	nd	nd	nd
109	PFL2310w	27	5	0.172	17	10	0.217	nd	nd	nd
113	PF13_0088	22	14	0.167	21	5	0.122	nd	nd	nd
43	PFB0765w	27	8	0.161	17	7	0.143	nd	nd	nd
153	PFB0765w	57	32	0.144	62	27	0.154	nd	nd	nd
48	PFC0760c	24	14	0.188	21	5	0.147	nd	nd	nd
49		30	11	0.127	67	45	0.201	26	3	0.103

59	PFE0100w	24	14	0.220	33	2	0.177	nd	nd	nd
110	PFL2520w	8	3	0.140	14	0	0.146	nd	nd	nd
124	MAL13P1.304	57	27	0.353	50	36	0.259	51	10	0.136
78	PF08_0048	54	43	0.309	79	4%	0.359	51	36	0.192
135	PF07_0021	41	14	0.159	53	20	0.143	nd	nd	nd
136	PFB0315w	76	51	0.176	93	65	0.406	54	13	0.109
137	MAL8P1.12	89	57	0.297	80	48	0.246	33	5	0.101
138	PF07_0086	89	51	0.352	68	55	0.378	15	21	0.100
139	MAL13P1.96	27	14	0.144	70	48	0.218	nd	nd	nd
140	PFC0345w	51	46	0.302	75	73	0.505	15	5	0.107
141	PFL0115w	51	11	0.107	38	8	0.097	nd	nd	nd
142	PFL0350c	32	24	0.240	75	43	0.163	nd	nd	nd
143	PFL1930w	3	5	0.129	8	3	0.097	nd	nd	nd
144		43	11	0.107	28	3	0.105	nd	nd	nd
145	PF13_0120	30	8	0.099	35	8	0.114	nd	nd	nd
146	PF13_0239	38	5	0.100	20	3	0.089	nd	nd	nd
147	PFD0520c	59	41	0.401	88	53	0.406	28	10	0.128
148	PF07_0111	19	3	0.129	20	0	0.130	nd	nd	nd
149	MAL6P1.254	41	5	0.127	30	19	0.198	nd	nd	nd
150	PF11_0424	38	8	0.131	50	30	0.203	nd	nd	nd
151	PFD0970c	43	43	0.200	78	50	0.319	10	3	0.107
152	PF14_0574	35	22	0.216	70	38	0.188	nd	nd	nd
133	PF14_0089	62	59	0.570	100	98	0.825	85	74	0.583
159	PFD0520c	59	30	0.201	40	23	0.198	nd	nd	nd
160	PFD0520c	62	46	0.290	70	35	0.239	nd	nd	nd
161	PFD0520c	92	68	0.520	88	73	0.496	74	49	0.462
162	PFD0520c	86	54	0.447	88	78	0.689	46	28	0.354
163	PFD0520c	46	24	0.193	90	83	0.650	33	23	0.284

Thus, 71 new proteins were identified with length varying from 200 to 10,000 amino acids. Two of these proteins (protein PF14_0089; PFD0520c) containing antigenic peptide (SEQ ID N°38; 147) were chemically synthesized as a single or overlapping polypeptides and tested for recognition in ELISA assays (see Table 2). These novel protein fragments are recognized by 15-100% of adult sera from Burkina Faso, Tanzania and Colombia. Affinity purified antibodies recognize parasite infected red blood cells. In addition, the fragment derived from the protein PF14_0089 has also been associated with protection in Tanzanian children (data not shown).

“Biologically active fragments” refer to a part of a sequence containing less amino acids in length than the sequence of the peptide of the invention. This sequence can be used as long as it exhibits the same properties as the native sequence from which it derives. Preferably this sequence contains less than 30%, preferably less than 60%, in particular less than 90% amino acids in length than the respective sequence of the peptide of the invention. Preferably also these sequences contain at least 20, most preferably 25, more preferably 40 and even more preferably 50 contiguous amino acids in length in common with sequence of the peptide of the invention.

These biologically active fragments can be prepared by a variety of methods and techniques known in the art such as for example chemical synthesis (*e.g.* multi-channel peptide synthesizer).

Furthermore, since an inherent problem with native peptides (in L-form) is the degradation by natural proteases, the peptide of the invention may be prepared in order to include D-forms and/or "retro-inverso isomers" of the peptide. Preferably, retro-inverso isomers of short parts, variants or combinations of the peptide of the invention are prepared.

By "retro-inverso isomer" is meant an isomer of a linear peptide in which the direction of the sequence is reversed and the chirality of each amino acid residue is inverted; thus, there can be no end-group complementarity.

Protecting the peptide from natural proteolysis should therefore increase the effectiveness of the specific heterobivalent or heteromultivalent compound. A higher biological activity is predicted for the retro-inverso containing peptide when compared to the non-retro-inverso containing analog owing to protection from degradation by native proteinases. Furthermore they have been shown to exhibit an increased stability and lower immunogenicity [Sela M.

and Zisman E., (1997) Different roles of D-amino acids in immune phenomena- FASEB J. 11, 449].

5 Retro-inverso peptides are prepared for peptides of known sequence as described for example in Sela and Zisman, (1997).

Also encompassed by the present invention are modifications of the antigenic peptide (which do not normally alter primary sequence), including in vivo or in vitro chemical derivitization of peptides, e.g., acetylation or carboxylation. Also included are modifications of
10 glycosylation, *e. g.*, those made by modifying the glycosylation patterns of a peptide during its synthesis and processing or in further processing steps, *e. g.*, by exposing the peptide to enzymes which affect glycosylation *e. g.*, mammalian glycosylating or deglycosylating enzymes. Also included are sequences which have phosphorylated amino acid residues, *e. g.*, phosphotyrosine, phosphoserine, or phosphothreonine.

15 Encompassed by the present invention is also a molecular chimera of the antigenic peptide of the invention. By "molecular chimera" is intended a polynucleotide or polypeptide sequence that may include a functional portion of the antigenic peptide and that will be
20 obtained, for example, by protein chemistry techniques known by those skilled in the art.

Particular combinations of the antigenic peptide sequence or fragments or subportions thereof are also considered in the present invention. Preferably, as disclosed in example 4, the combination antigenic peptide is selected from the group comprising the peptide LR162 (SEQ
25 ID N° 154), LR162A (SEQ ID N° 155), LR179 (SEQ ID N° 156), LR179A (SEQ ID N° 157) and LR181 (SEQ ID N° 158).

The present invention also includes variants of the antigenic peptide. The term
"variants" refer to polypeptides having amino acid sequences that differ to some extent from a
30 native sequence polypeptide, that is amino acid sequences that vary from the native 3D sequence whereby one or more amino acids are substituted by another one. The variants can occur naturally (*e.g.* polymorphism) or can be synthesized. Variants possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence of the native

amino acid sequence. Amino acid substitutions are herein defined as exchanges within one of the following five groups:

- I. Small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, Gly
 - 5 II. Polar, positively charged residues: His, Arg, Lys
 - III. Polar, negatively charged residues: and their amides: Asp, Asn, Glu, Gln
 - IV. Large, aromatic residues: Phe, Tyr, Trp
 - V. Large, aliphatic, nonpolar residues: Met, Leu, Ile, Val, Cys.
- 10 Non-natural amino acids can also be introduced by chemical synthesis.

The present invention also relates to an antigenic cocktail composition of Plasmodium species that comprises at least 2 antigenic peptides of the invention or mixtures thereof.

Applicants have shown that the use of an antigenic cocktail composition of the invention
 15 improves the performance of vaccines since simultaneous immune responses to antigens, which are involved in the same or in different pathogenic mechanisms, surprisingly confers greater and sustained protection. The antigenic peptides may be free or linked together. In case said antigenic peptides are linked, they are preferably linked together by the way of a linker such as PEG (Poly ethylene glycol).

20 Preferably, the antigenic cocktail composition is selected from the group comprising a combination with SEQ ID N° 1, 2 and 3, or SEQ ID N° 16, 33, 38 and 2 or SEQ ID N° 35, 27, 4, 34, 24, and 37.

Also encompassed by the present invention is the use of an antigenic peptide or of the
 25 antigenic cocktail composition of the invention for the preparation of a vaccine composition useful to stimulate an immune response in a mammal. "Mammal" refers to any animal classified as a mammal including humans, domestic and farm animals, and zoo, sports or pet animals, such as dogs, horses, cats, cows, monkeys, etc. Preferably the mammal is a human.

30 To augment the immune response elicited, it may be preferable to couple the peptides of SEQ ID NOS: 1-163, or the antigenic cocktail composition, to any carrier molecule or carrier proteins. Various protein, glycoprotein, carbohydrate or sub-unit carriers can be used,

including but not limited to, tetanus toxoid/toxin, diphtheria toxoid/toxin, pseudomonas mutant carrier, bacteria outer membrane proteins, crystalline bacterial cell surface layers, various endo or exotoxins, serum albumin, gamma globulin or keyhole limpet hemocyanin, recombinant, exotoxin A, LT toxin, Cholera B toxin, Klebsiella pneumoniae OmpA, Bacterial flagella, Clostridium difficile recombinant toxin A, Peptide dendrimers (multiple antigenic peptides), pan DR epitope (PADRE), universal T-cell epitopes from tetanus toxin, Commensal bacteria, Phage (displaying peptide on and bacteria phages), attachment of peptides to recombinant IgG1 and/or other suitable constituents.

10 In addition, the peptides of SEQ ID NOS: 1-163, or the antigenic cocktail composition, or their conjugates with carrier proteins may be further mixed with adjuvants to elicit an immune response, as adjuvants may increase immunoprotective antibody titers or cell mediated immunity response. Such adjuvants can include, but are not limited to, MPL + TDM + CWS (SIGMA), MF59 (an oil-in-water emulsion that includes 5% squalene, 0.5% sorbitan monoleate and 0.5% sorbitan trioleate Chiron), Heat-labile toxin (HLT), CRMig (nontoxic genetic mutant of diphtheria toxin), Squalene (IDEC PHARMACEUTICALS CORP.), Ovalbumin (SIGMA), Quil A (SARGEANT, INC.), Aluminum phosphate gel (SUPERFOS BIOSECTOR), Cholera holotoxin (CT LIST BIOLOGICAL LAB.), Cholera toxin B subunit (CTB), Cholera toxin A subunit- Protein A D-fragment fusion protein, Muramyl dipeptide (MDP), Adjumera (polyphosphazene, VIRUS RESEARCH INSTITUTE), SPT (an emulsion of 5% squalene, 0.2% Tween 80, 1.25% Pluronic L121 with phosphate-buffered saline pH 7.4), Avridine (M6 PHARMACEUTICALS), Bay R1005 (BAYER), Calcitrol (SIGMA), Calcium phosphate gel (SARGEANT INC.), CRL 1005 (Block co-polymer P1205, VAXCEL CORP.), DHEA (MERCK), DMPC (GENZYME PHARMACEUTICALS and FINE CHEMICALS). DMPG (GENZYME PHARMACEUTICALS and FINE CHEMICALS), Gamma Inulin, Gerbu Adjuvant (CC BIOTECH CORP.), GM-CSF, (IMMUNE CORP.), GMDP (PEPTECH LIMITED), Imiquimod (3M PHARMACEUTICALS), ImmTher (ENDOREX CORPORATION), ISCOMTM (ISCOTEC AB), Iscoprep 7.0.3 TM (ISCOTEC AB), Loxoribine, LT-Oral Adjuvant (E. coli labile enterotoxin, protoxin, BERNA PRODUCTS CORP.), MTP-PE (CIBA-GEIGY LTD), Murametide, (VACSYN S. A.), Murapalmitine (VACSYN S. A.), Pluronic L121 (IDEC PHARMACEUTICALS CORP.), PMMA (INSTITUT FUR PHARMAZEUTISCHE TECHNOLOGIE), SAF-1 (SYNTEX

ADJUVANT FORMULATION CHIRON), Stearyl tyrosine (BIOCHEM THERAPEUTIC INC.), Theramidea (IMMUNO THERAPEUTICS INC.), Threonyl- MDP (CHIRON), FREUNDS complete adjuvant, FREUNDS incomplete adjuvant, aluminum hydroxide, dimethyldioctadecyl-ammonium bromide, Adjuvax (ALPHA- BETA TECHNOLOGY), Inject Alum (PIERCE), Monophosphoryl Lipid A (RIBI IMMUNOCHEM RESEARCH), MPL+TDM (RIBI IMMUNOCHEM RESEARCH), Titermax (CYTRX), QS21, t Ribi Adjuvant System, TiterMaxGold, QS21, Adjumer, Calcitrol, CTB, LT (E. coli toxin), LPS (lipopolysaccharide), Avridine, the CpG sequences (Singh et al. , 1999 Singh, M. and Hagum, D., Nature Biotechnology 1999 17: 1075-81) toxins, toxoids, glycoproteins, lipids, glycolipids, bacterial cell walls, subunits (bacterial or viral), carbohydrate moieties (mono-, di-, tri-, tetra-, oligo-and polysaccharide), various liposome formulations or saponins. Combinations of various adjuvants may be used with the antigen to prepare the immunogen formulations. Adjuvants administered parentally or for the induction of mucosal immunity may also be used.

The present invention also contemplates a vaccine composition useful to stimulate an immune response in a mammal characterized in that it comprises an antigenic peptide, or an antigenic cocktail composition.

The vaccine composition can be administered by various delivery methods including intravascularly, intraperitoneally, intramuscularly, intradermally, subcutaneously, orally, nasally or by inhalation. In an embodiment, the compositions can further include a pharmaceutically acceptable excipient and/or carrier.

“Administered” or “administering”, as it applies in the present invention, refers to contact of a pharmaceutical, therapeutic, diagnostic agent or composition, to the subject, preferably a human.

The exact formulation of the vaccine composition will depend on the particular antigenic peptide, or an antigenic cocktail, or peptide- carrier conjugate, and the route of administration.

When employing more than one antigenic peptide, such two or more, or an antigenic cocktail composition, they may be used as a physical mixture or a fusion of two or more antigenic peptides to form a combination. The combination may be produced, for example, by

recombinant techniques, by the use of appropriate linkers for fusing previously prepared antigenic peptides or by co-linearly synthesizing the combination with or without linkers such as, for example, PEG (poly ethylene glycol).

5 Also encompassed in the present invention is an antibody characterized in that it recognizes the antigenic peptide of the invention or the antigenic cocktail composition of the invention.

As used herein, an "antibody" is a protein molecule that reacts with a specific antigen and belongs to one or five distinct classes based on structural properties: IgA, IgD, IgE, IgG
10 and IgM. The antibody can be monoclonal or polyclonal.

The antibodies of the invention can also be produced for use in passive immunotherapy, for use as diagnostic reagents, and for use as reagents in other processes such as affinity chromatography.

When used in passive immunotherapy, the antibody of the invention can be included in a
15 pharmaceutical composition and administered to a mammal. In an embodiment, the pharmaceutical composition includes a pharmaceutically acceptable carrier, and optionally can include pharmaceutically acceptable excipients. The pharmaceutical composition can be administered intravascularly, intraperitoneally, intramuscularly, intradermally, subcutaneously, orally, nasally or by aerosol inhalation. Preferably, the pharmaceutical composition is
20 administered intravascularly, intramuscularly, orally, nasally or by aerosol inhalation.

In an embodiment, the present invention includes an antibody, particularly a monoclonal antibodies, directed to the antigenic peptide or the antigenic cocktail composition of the invention. In particular, hybridomas can be generated using a peptide of SEQ ID NOS:
25 1-163 and recombinant derivative antibodies can be made using these hybridomas according to well-known genetic engineering methods (Winter G. , and Milstein C., Nature, 1991,349 293-299). Preferably, as disclosed in example 2 and Table 3, the antibody is selected from the group comprising α -8, α -9, α -11/12, α -13, α -14, α -27, α -86, α -102, α -99, α -68, α -41, α -110, α -78, α -135, α -136, α -138, α -146 and α -151 or mixtures thereof.

30 Other methods known in the art to humanize an antibody or produce a humanized antibody can be utilized as well. These methods can include but are not limited to the xenomouse technology developed by ABGENIX INC. (See, U. S. Patent Nos. 6,075, 181 and

6,150, 584) and the methods developed by BIOVATION, BIOINVENT INTERNATIONAL AB, PROTEIN DESIGN LABS, APPLIED MOLECULAR EVOLUTION, INC. , IMMGENICS PHARMACEUTICALS INC., MEDAREX INC. , CAMBRIDGE ANTIBODY TECHNOLOGY, ELAN, EOS BIOTECHNOLOGY, MEDIMMUNE, MORPHOSYS, UROGENSYS INC. , AVANIR PHARMACEUTICAL/XENEREX BIOSCIENCES, AFFIBODY AB, ALLEXION ANTIBODY TECHNOLOGIES, ARIUS RESEARCH INC., CELL TECH, XOMA, IDEC PHARMACEUTICALS, NEUGENESIS, EPICYTE, SEMBIOSYS GENETICS INC., BIOPROTEIN, GENZYME THERAPEUTICS, KIRIN, GEMINI SCIENCES, HEMATECH.

Likewise, other methods known in the art to screen human antibody secreting cells to SEQ ID NOS: 1-163 may be also be utilized.

As used herein, the term "humanized antibody" or other like terms means an antibody that includes a human protein sequence in at least a portion thereof. The amount of human protein sequence can vary depending on how the antibody is made.

A "fully humanized antibody" or "human antibody" as the terms or like terms are used herein can be made, for example, with xenomouse technology as discussed above or transforming human B cells with Epstein Barr virus (Traggiai et al, 2004 in Nat. Med. 10(8): 871-5). Other methods such as phage display techniques are also possible (Bradbury AR, Marks JD, 2004 in J. immunol. Methods, 290 (1-2): 29-49).

The term "recognize" refers to the fact that an antibody of the invention is directed to an antigenic peptide or an antigenic cocktail composition of the invention and binds thereto.

When recombinant techniques are employed to prepare an antigenic peptide in accordance with the present invention, nucleic acid molecules or fragments thereof encoding the polypeptides are preferably used.

Therefore the present invention also relates to a purified and isolated nucleic acid sequence comprising

- i) a nucleotide sequence encoding an antigenic peptide of the invention,

- ii) a nucleic acid sequence complementary to i),
- iii) a degenerated nucleic acid sequence of i) or ii),
- iv) a nucleic acid sequence capable of hybridizing under stringent conditions to i), ii) or iii),
- 5 v) a nucleic acid sequence encoding a truncation or an analog of an antigenic peptide of the invention,
- vi) and/or a fragment of i), ii), iii), iv) or v) encoding a biologically active fragment of of said antigenic peptide of the invention.

10 “A purified and isolated nucleic acid sequence” refers to the state in which the nucleic acid molecule encoding the antigenic peptide the invention, or nucleic acid encoding such the antigenic peptide will be, in accordance with the present invention. Nucleic acid will be free or substantially free of material with which it is naturally associated such as other polypeptides or nucleic acids with which it is found in its natural environment, or the
 15 environment in which it is prepared (e. g. cell culture) when such preparation is by recombinant nucleic acid technology practised *in vitro* or *in vivo*.

The term “nucleic acid” is intended to refer either to DNA or to RNA.

20 In case the nucleic acid is DNA, then DNA which can be used herein is any polydeoxynucleotide sequence, including, e.g. double-stranded DNA, single-stranded DNA, double-stranded DNA wherein one or both strands are composed of two or more fragments, double-stranded DNA wherein one or both strands have an uninterrupted phosphodiester backbone, DNA containing one or more single-stranded portion(s) and one or more double-
 25 stranded portion(s), double-stranded DNA wherein the DNA strands are fully complementary, double-stranded DNA wherein the DNA strands are only partially complementary, circular DNA, covalently- closed DNA, linear DNA, covalently cross-linked DNA, cDNA, chemically- synthesized DNA, semi-synthetic DNA, biosynthetic DNA, naturally-isolated DNA, enzyme-digested DNA, sheared DNA, labeled DNA, such as radiolabeled DNA and
 30 fluorochrome-labeled DNA, DNA containing one or more non-naturally occurring species of nucleic acid.

DNA sequences that encode the antigenic peptide of the invention, or a fragment thereof, can be synthesized by standard chemical techniques, for example, the phosphotriester method or via automated synthesis methods and PCR methods.

The purified and isolated DNA sequence encoding the antigenic peptide according to the invention may also be produced by enzymatic techniques. Thus, restriction enzymes, which cleave nucleic acid molecules at predefined recognition sequences can be used to isolate nucleic acid sequences from larger nucleic acid molecules containing the nucleic acid sequence, such as DNA (or RNA) that codes for the antigenic peptide of the invention or for a fragment thereof.

Encompassed by the present invention is also a nucleic acid in the form of a polyribonucleotide (RNA), including, *e.g.*, single-stranded RNA, double-stranded RNA, double-stranded RNA wherein one or both strands are composed of two or more fragments, double-stranded RNA wherein one or both strands have an uninterrupted phosphodiester backbone, RNA containing one or more single-stranded portion(s) and one or more double-stranded portion(s), double-stranded RNA wherein the RNA strands are fully complementary, double-stranded RNA wherein the RNA strands are only partially complementary, covalently crosslinked RNA, enzyme-digested RNA, sheared RNA, mRNA, chemically-synthesized RNA, semi-synthetic RNA, biosynthetic RNA, naturally-isolated RNA, labeled RNA, such as radiolabeled RNA and fluorochrome-labeled RNA, RNA containing one or more non-naturally- occurring species of nucleic acid.

The purified and isolated nucleic acid sequence, DNA or RNA, also comprises a purified and isolated nucleic acid sequence having substantial sequence identity or homology to a nucleic acid sequence encoding an antigenic peptide of the invention. Preferably, the nucleic acid will have substantial sequence identity for example at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 85% nucleic acid identity; more preferably 90% nucleic acid identity; and most preferably at least 95%, 96%, 97%, 98%, or 99% sequence identity.

"Identity" as known in the art and used herein, is a relationship between two or more amino acid sequences or two or more nucleic acid sequences, as determined by comparing the sequences. It also refers to the degree of sequence relatedness between amino acid or nucleic

acid sequences, as the case may be, as determined by the match between strings of such sequences. Identity and similarity are well known terms to skilled artisans and they can be calculated by conventional methods (for example see Computational Molecular Biology, Lesk, A. M. ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W. ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M. and Griffin, H. G. eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G. Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J. eds. M. Stockton Press, New York, 1991, Carillo, H. and Lipman, D., SIAM J. Applied Math. 48:1073, 1988).

Methods which are designed to give the largest match between the sequences are generally preferred. Methods to determine identity and similarity are codified in publicly available computer programs including the GCG program package (Devereux J. et al., Nucleic Acids Research 12(1): 387, 1984); BLASTP, BLASTN, and FASTA (Atschul, S. F. et al. J. Molec. Biol. 215: 403-410, 1990). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S. et al. NCBI NLM NIH Bethesda, Md. 20894; Altschul, S. et al. J. Mol. Biol. 215: 403-410, 1990).

Also encompassed by the present invention is a nucleic acid sequence complementary to the antigenic peptide of the invention.

20

Also within the scope of the invention is a degenerated nucleic acid sequence having a sequence which differs from a nucleic acid sequence encoding the antigenic peptide of the invention, or a complementary sequence thereof, due to degeneracy in the genetic code. Such nucleic acid encodes functionally equivalent to the antigenic peptide of the invention but differs in sequence from the sequence due to degeneracy in the genetic code. This may result in silent mutations which do not affect the amino acid sequence. Any and all such nucleic acid variations are within the scope of the invention.

25

In addition, also considered is a nucleic acid sequence capable of hybridizing under stringent conditions, preferably high stringency conditions, to a nucleic acid sequence encoding the antigenic peptide of the invention, a nucleic acid sequence complementary thereof or a degenerated nucleic acid sequence thereof. Appropriate stringency conditions

30

which promote DNA hybridization are known to those skilled in the art, or can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, 6.0X sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0XSSC at 50°C may be employed. The stringency may be selected based on the conditions used in the wash step. By way of example, the salt concentration in the wash step can be selected from a high stringency of about 0.2XSSC at 50°C. In addition, the temperature in the wash step can be at high stringency conditions, at about 65° C.

The present invention also includes a purified and isolated nucleic acid encoding an antigenic peptide of the invention comprising a nucleic acid sequence encoding a truncation or an analog of the antigenic peptide.

The invention also encompasses allelic variants of the disclosed purified and isolated nucleic sequence; that is, naturally-occurring alternative forms of the isolated and purified nucleic acid that also encode antigenic peptides that are identical, homologous or related to that encoded by the purified and isolated nucleic sequences. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

A fragment of the disclosed purified and isolated nucleic sequence is also considered and refers to a sequence containing less nucleotides in length than the nucleic acid sequence encoding the antigenic peptide, a nucleic acid sequence complementary thereof or a degenerated nucleic acid sequence thereof. This sequence can be used as long as it exhibits the same properties as the native sequence from which it derives. Preferably this sequence contains less than 90%, preferably less than 60%, in particular less than 30% amino acids in length than the respective purified and isolated nucleic sequence of the antigenic peptide.

Yet another concern of the present invention is to provide an expression vector comprising at least one copy of the purified and isolated nucleic acid sequence encoding an antigenic peptide of the invention as described above.

The choice of an expression vector depends directly, as it is well known in the art, on the functional properties desired, *e.g.*, antigenic peptide expression and the host cell to be transformed or transfected.

5 Additionally, the expression vector may further comprise a promoter operably linked to the purified and isolated nucleic acid sequence of the invention. This means that the linked isolated and purified nucleic acid sequence encoding the antigenic peptide of the present invention is under control of a suitable regulatory sequence which allows expression, *i.e.* transcription and translation of the inserted isolated and purified nucleic acid sequence.

10 As used herein, the term “promoter” designates any additional regulatory sequences as known in the art *e.g.* a promoter and/or an enhancer, polyadenylation sites and splice junctions usually employed for the expression of the polypeptide or may include additionally one or more separate targeting sequences and may optionally encode a selectable marker. Promoters which can be used provided that such promoters are compatible with the host cell are *e.g.*
15 promoters obtained from the genomes of viruses such as polyoma virus, adenovirus (such as Adenovirus 2), papilloma virus (such as bovine papilloma virus), avian sarcoma virus, cytomegalovirus (such as murine or human cytomegalovirus immediate early promoter), a retrovirus, hepatitis-B virus, and Simian Virus 40 (such as SV 40 early and late promoters) or promoters obtained from heterologous mammalian promoters, such as the actin promoter or an
20 immunoglobulin promoter or heat shock promoters.

Enhancers which can be used are *e.g.* enhancer sequences known from mammalian genes (globin, elastase, albumin, α -fetoprotein, and insulin) or enhancer from a eukaryotic cell virus *e.g.* the SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma, and
25 adenovirus enhancers.

A wide variety of host/expression vector combinations may be employed in expressing the nucleic acid sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences.

30 Suitable vectors include derivatives of SV40 and known bacterial plasmids, *e.g.*, *E. coli* plasmids col El, pCRI, pBR322, pMB9 and their derivatives, plasmids such as RP4; phage DNAs, *e.g.*, the numerous derivatives of phage X, *e.g.*, NM989, and other phage DNA, *e.g.*,

M13 and filamentous single stranded phage DNA; yeast plasmids such as the 2 μ plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control
5 sequences; and the like.

Another concern of the present invention is to provide a host cell (eukaryotic or prokaryotic) comprising a purified and isolated nucleic acid sequence of the invention or an expression vector as described above.

10 Typically, this host cell has been transformed or transfected with a purified and isolated nucleic acid sequence of the invention or an expression vector described herein.

The term "cell transfected" or "cell transformed" or "transfected/transformed cell" means the cell into which the extracellular DNA has been introduced and thus harbours the extracellular DNA. The DNA might be introduced into the cell so that the nucleic acid is
15 replicable either as a chromosomal integrant or as an extra chromosomal element.

Transformation or transfection of appropriate eukaryotic or prokaryotic host cells with an expression vector comprising a purified an isolated DNA sequence according to the invention is accomplished by well known methods that typically depend on the type of vector
20 used. With regard to these methods, see *e.g.*, *Maniatis et al. 1982*, Molecular Cloning, A laboratory Manual, Cold Spring Harbor Laboratory and commercially available methods.

A wide variety of unicellular host cells are useful in expressing the nucleic acid sequences of this invention. These hosts may include well known eukaryotic and prokaryotic
25 hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, fungi such as yeasts, and animal cells, such as CHO, YB/20, NSO, SP2/0, R1. 1, B-W and L-M cells, African Green Monkey kidney cells (*e. g.*, COS 1, COS 7, BSC1, BSC40, and BMT10), insect cells (*e. g.*, Sf9), and human cells and plant cells in tissue culture. Preferably, the host cell is a bacterial cell, more preferably an *E. coli* cell.

The present invention is also directed to a vaccine composition for the treatment and/or prevention of malaria comprising at least the antigenic peptide of the invention, or the antigenic cocktail composition of the invention.

5 In a further aspect, the present invention is also directed to an acid nuclei vaccine composition for the treatment and/or prevention of malaria comprising at least a purified and isolated nucleic acid sequence of the invention, or an expression vector comprising at least one copy of the purified and isolated nucleic acid sequence of the invention, fragments thereof, molecular chimeras thereof, combinations thereof and/or variants thereof.

10 Yet another aspect of the present invention is a method of producing an antigenic peptide for the preparation of a vaccine composition against Plasmodium species, characterized in that it comprises the steps of:

- 15 a) identifying the presence of a sequence encoding at least one coiled coil region in the genome of said Plasmodium species,
- b) selecting said sequence encoding at least one coiled coil region,
- c) synthesizing an antigenic peptide corresponding to the sequence containing the at least one coiled coil region selected in step c),
- 20 d) contacting the synthesized antigenic peptide with a serum sample from at least one donor living in pathogen endemic areas; and
- e) determining whether at least one antibody of said serum sample binds to the synthesized antigenic peptide.

25 Identification of the presence of a sequence encoding at least one coiled coil region in the genome of said Plasmodium species can be performed by a bioinformatics tool such as computer programs based on algorithms.

30 For example, Plasmodium falciparum 3D7 genome was used for the bioinformatics analysis (Gardner, 2002). Software named *pftools* and containing programs for sensitive generalized sequence profiles (Bucher et al., 1996) was used to search for the short α -helical coiled coil regions. The coiled coil profiles were constructed using an alignment of amino acid sequences corresponding to the known coiled coil domain. Two profiles containing four and

five heptad repeats were used for the analysis. The coiled coil regions selected by this approach were also tested by the COILS program.

The selected α -helical coiled coil containing proteins were further tested on their possible surface location and GPI anchoring by using the following programs: identification of potential signal peptides (PSORT, <http://www.psorth.org/psorth/>, SignalP, <http://www.cbs.dtu.dk/services/SignalP/>), transmembrane spanning regions (TMPRED http://www.ch.embnet.org/software/TMPRED_form.html and TMHMM <http://www.cbs.dtu.dk/services/TMHMM/>), and GPI-anchored proteins http://mendel.imp.univie.ac.at/sat/gpi/gpi_server.html.

The same computer program, or another one, will then compare the selected sequence to the transcriptome and the proteome databases of said pathogen in order to, for example, identify proteins in the asexual erythrocytic stages (www.PlasmoDB.org). If the coiled coil score of this sequence is high and if it is present in both the transcriptome and the proteome databases, it will be selected for subsequent peptide synthesis.

Peptide synthesis is performed according to techniques well known in the art such as solid-phase Fmoc chemistry. Usually the crude peptide is then purified by RP-HPLC or any other purification techniques, and analyzed by mass spectrometry (MALDI-TOF).

Synthetic peptide is then contacted with one serum, or a panel of sera, from individuals living in malaria endemic areas, and which have been infected by the pathogen, to assess if said serum/sera bind to the synthesized antigen, and thus underlying a positive antibody response. These responses will be further analysed for the presence of IgG1 and IgG3 subclasses, which have been shown, in case the pathogen is *Plasmodium falciparum*, to mediate protection by the reduction of *Pf* growth by an antibody dependant cytotoxic inhibition (ADCI) mechanism (Oeuvery et al, 1994). Biological activity of antibodies will be tested in ADCI and invasion inhibition assays (Okoyeh et al, 1999, Haynes et al, 2002) using peptide specific antibodies purified by affinity chromatography.

Usually the pathogen will be selected among the group comprising *Mycobacterium tuberculosis*, Influenza, *Toxoplasma*, Ebola virus, *Streptococcus*, *Plasmodium falciparum*, *meningococcus* and *staphylococcus*

In case the pathogen is *Plasmodium falciparum*, the proteome and transcriptome data available in the literature (Betts, 2002, Florens et al, 2002, Bozdech et al, 2003) also allow for assessing whether these molecules are present in the erythrocytic stage .

5 The present invention also encompasses a diagnostic tool for determining the presence of an antigenic peptide or an antigenic cocktail composition of the invention in a sample comprising:

i) contacting the sample with an antibody directed to the antigenic peptide or the antigenic cocktail composition of the invention, and

10 ii) determining whether said antibody binds to a component of said sample.

The present invention further encompasses a diagnostic tool for determining the presence of antibodies directed to the antigenic peptide or to the antigenic cocktail composition of the invention in a sample comprising:

15 i) contacting said sample with the antigenic peptide or the antigenic cocktail composition of the invention, and

ii) determining whether antibodies bind to a component of said antigenic peptide or to said antigenic cocktail composition of the invention.

20 As used herein, "a sample" is an aliquot or a representative portion of a substance, material or population. For example, a sample may be a sample of blood, biological tissue, urine or feces. Preferably, the sample is blood.

As used herein, "a donor" is an individual person that lives in pathogen endemic areas and that has been, or is suspected to be, infected by said pathogen.

25 A "component" refers to any amino acid, nucleic acid, lipid or motif that is recognized by the antibody of the invention.

Also encompassed by the present invention is a protein that comprises at least one antigenic peptide deriving from *Plasmodium* species, said antigenic peptide comprising at least one coiled coil region.

30 Preferably the protein comprises sequences selected from the group comprising the amino acid sequences SEQ ID N°1 to 132, SEQ ID N°134 to SEQ ID N°153 and SEQ ID

N°159 to SEQ ID N°163, biologically active fragments thereof, molecular chimeras thereof, combinations thereof and/or variants thereof. Most preferably the protein is PF14_0089 (containing SEQ ID N° 38) or protein PFD0520c.

Usually the protein containing at least one antigenic peptide of the invention will be chemically synthesized and tested for recognition in ELISA assays. However, any other recombinant technique or recovery method such as expressing the protein in a host cell from a nucleotide sequence encoding said protein is also considered.

The invention also encompassed a vaccine composition useful to stimulate an immune response in mammal comprising a protein containing at least one antigenic peptide of the invention and the use thereof.

Further encompassed in the present invention is an antibody that recognizes a protein containing at least one antigenic peptide of the invention and its use in diagnostic tools.

Also encompassed is a purified and isolated nucleic acid sequence comprising

- i) a nucleotide sequence encoding the protein of the invention,
- ii) a nucleic acid sequence complementary to i),
- iii) a degenerated nucleic acid sequence of i) or ii),
- iv) a nucleic acid sequence capable of hybridizing under stringent conditions to i), ii) or iii),
- v) a nucleic acid sequence encoding a truncation or an analog of the protein of the invention,
- vi) and/or a fragment of i), ii), iii), iv) or v).

The present invention also relates to an expression vector comprising at least one copy of said purified and isolated nucleic acid sequence encoding the protein of the invention, and a host cell comprising either said purified and isolated nucleic acid sequence or said expression vector.

Also within the scope of the present invention is a kit, said kit comprising the vaccine composition as described herein, optionally with reagents and/or instructions for use.

Alternatively, or additionally, the kit may further include other materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, and syringes.

- 5 Various references are cited throughout this Specification, each of which is incorporated herein by reference in its entirety.

The foregoing description will be more fully understood with reference to the following Examples. Such Examples, are, however, exemplary of methods of practising the present

- 10 invention and are not intended to limit the scope of the invention.

EXAMPLES

Example 1

Materials

Chemicals and solvents used for the synthesis were purchased from Fluka (Buchs, Switzerland) and Novabiochem (Laufelfinger, Switzerland). Pooled human sera from Tanzania, individual adult sera from Burkina Faso and Tanzania and merozoite slides were obtained from Swiss Tropical Institute (STI, Basel, Switzerland). Sporozoite slides were obtained from Nijmegen (Holland).

Peptide Synthesis

All the peptides were synthesized at the Department of Biochemistry (University of Lausanne, Switzerland) using solid-phase Fmoc chemistry using the Advanced ChemTech (Hatley St George, UK) AC T348 Omega multi channel synthesizer and the Applied Biosystem synthesizer 431A, Foster City, CA). Protein PF14_0089 was synthesized from residue 47 to 186 since the 1-46 fragment was predicted to contain a leader and a trans-membrane sequence (plasmodb.org; September 2004). Crude peptides were purified by RP-HPLC (C₁₈ preparative column) and analyzed by mass spectrometry (MALDI-TOF; Applied Biosystem).

ELISA

Microtitre 96-well plates (Maxisorp F96, Nunc, Denmark) were coated overnight (O/N) at 4°C with 50 µl/well of peptide at a concentration of 5 µg/ml. Plates were washed using phosphate buffered saline solution containing 0.05% Tween 20 (PBS-T; Sigma, St-Louis, MO, USA) and saturated with 5% non-fat dry milk in PBS-T for 1 hour at room temperature (RT). Serial dilutions of individual mice sera or individual human sera diluted at 1/200 in PBS-T-2.5% non-fat dry milk (PBS-T-milk) were added into the plates and incubated for 1 hour at RT. After washing, goat anti-mouse or human polyvalent immunoglobulin conjugated to alkaline phosphatase (Sigma, St-Louis, MO, USA) diluted (1/1000) in PBS-T-milk was added and the plates incubated for 1 hour. Plates were washed and the presence of enzyme evidenced by the p-nitrophenylphosphate substrate (Sigma, St-Louis, MO, USA). Absorbance was measured at 405 nm with a Multiskan Ascent Reader. The test sample titre was determined as the highest dilution above the mean optical density (OD) value + 3 standard

deviation (SD) of the negative control (normal mouse serum or human serum from naïve donors).

5 Example 2: *Specific Antibody Purification by Affinity Chromatography*

Antigen-Sepharose conjugate preparation

5 mg of antigen was dissolved in 1 mL of coupling buffer (0.1 M NaHCO₃ containing 0.5 M NaCl, pH 8.0). The CNBr-sepharose 4B (Amersham Bioscience AB, Uppsala, Sweden) was
10 swollen in 1 mM HCl then the gel was washed with coupling buffer. The antigen solution was added to the gel and the mixture is stirred 1h at RT. After the coupling reaction, excess of antigen was washed away with coupling buffer. The remaining activated groups were blocked by treatment with ethanolamine (0.25 M; pH 8.0) for 30 min at RT. The gel was then washed with sodium acetate buffer (0.1 M; pH 4.0), followed by coupling buffer. The antigen-
15 sepharose conjugate was either used or stored at 4°C in PBS (1x) containing 1 mM azide.

Isolation of specific antibody

Pooled human sera was diluted four times with PBS (1x) containing 0.5 M sodium chloride and mixed with antigen-sepharose conjugate. This mixture was then stirred gently on a wheel
20 O/N at 4°C. After centrifugation human sera were collected and stored at -20°C until further used. The antigen-sepharose conjugate was then washed with 5 mL of trizma base TRIS (20 mM containing 0.5 M NaCl, pH 8.0) then with 5 mL of TRIS (20 mM, pH 8.0). The elution of bound antibody was achieved with glycine (0.1 M, pH 2.5). The fractions obtained were neutralized with TRIS (1 M, pH 8).

25

Indirect Fluorescence Antibody Test (IFAT)

Slides coated with *Pf* sporozoites were dried at RT for 30 minutes, fixed with 100% acetone at 4°C for 10 minutes, washed 2 times in PBS-T, dried carefully and blocked with 20 µL/well of PBS 5% fetal calf serum (FCS) for 30 minutes. Slides coated with *Pf* merozoites were fixed
30 with 100% acetone at -20°C for 15 minutes and dried O/N at RT. The appropriate antibody or serum dilutions prepared in PBS 1x-2.5% non-fat dry milk, were distributed (10 µL/well) and incubated 1h at RT (sporozoite) or 30 minutes at 37°C (merozoite) in a humid chamber. After washings with PBS-T, goat anti-mouse polyvalent immunoglobulin conjugated with Alexa

fluor 488 (Molecular Probes, Oregon, USA) diluted 1/50 in Evans blue solution (1/50000) was added (400µL/slide) and incubated 50 minutes at RT (sporozoite) or 30 minutes at 37⁰C (merozoite) in a humid chamber in the dark. After washings slides are covered with 50 % glycerol, sealed and read using Zeica 200 microscope.

5

Mice immunizations

Five- to seven-weeks old female CB6F1 mice were purchased from Harlan (Horst, Holland). Mice were injected subcutaneously at the base of the tail with 20 µg of peptide dissolved in PBS (1x) or water and emulsified in Montanide ISA720. For the mix of different peptides or the fragments of Mal6P1.37 and for the fragments of PfB0145c and PfD0110w, 10 and 5 µg/mouse respectively were injected. Animals received a booster dose of antigen after 3 and 6 weeks. Ten days after each boost, mice were bled to assess the presence of specific antibody by ELISA. If positive IFAT is performed, monoclonal antibodies are isolated according to Winter and Milstein (Nature, 1991,349 293-299). For results, see Table 7.

15

Indirect Fluorescence Antibody Test (IFAT)

Slides coated with *Pf* sporozoites were dried at RT for 30 minutes, fixed with 100% acetone at 4⁰C for 10 minutes, washed 2 times in PBS-T, dried carefully and blocked with 20 µL/well of PBS 5% fetal calf serum (FCS) for 30 minutes. Slides coated with *Pf* merozoites were fixed with 100% acetone at -20⁰C for 15 minutes and dried O/N at RT. The appropriate antibody or serum dilutions prepared in PBS 1x-2.5% non-fat dry milk, were distributed (10 µL/well) and incubated 1h at RT (sporozoite) or 30 minutes at 37⁰C (merozoite) in a humid chamber. After washings with PBS-T, goat anti-mouse polyvalent immunoglobulin conjugated with Alexa fluor 488 (Molecular Probes, Oregon, USA) diluted 1/50 in Evans blue solution (1/50000) or anti-human IgG (Fc specific) FITC conjugate (Sigma) was added (400µL/slide) and incubated 50 minutes at RT (sporozoite) or 30 minutes at 37⁰C (merozoite) in a humid chamber in the dark. After washings slides are covered with 50 % glycerol, sealed and read using Zeica 200 microscope.

Parasites

The Uganda Palo Alto strain (FUP/C) was cultured in RPMI-1640 supplemented with 0.5% albumax I (GibcoBRL-Invitrogen). For ADCI assays, blood stages parasite cultures were

synchronized by at least two successive sorbitol treatment followed, after maturation over 24 h, by floatation on 1% porcine skin gelatin type A (Sigma-Aldrich).

Human Sera

The sera from adults from Papua New Guinea (PNG) pooled for affinity purification were collected in the Maprik district of the East Sepik Province, during a cross sectional survey in July 1992 within the framework of the Malaria vaccine Epidemiology and Evaluation Project (MVEEP) supported by the United states Agency for International Development(5). The area is highly endemic for malaria. Ethical clearance for MVEEP was obtained from the PNG Medical Research Advisory Committee. Blood was taken by venipuncture into tubes containing EDTA.

The sera were collected in the village of Goundry located in the central Mossi Plateau, Burkina Faso, between 15 and 50 km north of the capital Ouagadougou, in the province of Oubritenga. The climate is characteristic of areas of Sudanese savannah, with a dry season from November to May and a rainy season from June to October. Malaria transmission is very high during the rainy season and markedly seasonal. The ethical clearance was obtained from the Ministry of Health, Burkina Faso. After obtaining informed consent from parents and caretakers, heparine venous blood samples were collected during a cross-sectional survey during the malaria low transmission season 1998.

Sera were collected in Buenaventura the main port on the Colombian Pacific Coast after human informed consent, during a cross sectional survey carried out from February to May 2002 within the framework of a project supported by the Colombian Research Council, COLCIENCIAS. The area has unestable transmission of both *P.falciparum* and *P.vivax* malaria. Ethical clearance to draw blood from human volunteers was obtained from the Institutional Review Board of Universidad del Valle. Blood was taken by venipuncture into tubes containing EDTA and sera fractionated and stored frozen until use.

The pool of immune African globulins (PIAG) was prepared from immune individuals living in endemic areas and negative control IgG (N-IgG) was obtained from a pool of more than 1000 French adult donors with no history of malaria. Briefly, the IgG fractions from both positive and negative controls were purified using a size exclusion Trisacryl GF05M (Pall BioSeptra) column followed by an ionic exchange DEAE Ceramic HyperD F column (Pall

BioSeptra). Purified IgG were then extensively dialyzed against RPMI and kept at 4°C until use.

Preparation of human blood monocytes

5 Blood monocytes (MN) were prepared from cytapheresis samples obtained from healthy blood donors with no previous history of malaria (Lecourbe Blood Bank, Paris, France). Peripheral blood mononuclear cells (PBMC) were separated on Ficoll density gradients (J Prep, Techgen) and washed in Ca^{2+} and Mg^{2+} free HBSS buffered with 10 mM HEPES (both from GibcoBRL-Invitrogen). Cells were then distributed on polystyrene 96-well flat-bottomed
10 culture plates (TPP, Switzerland) and adherent MN were selected by incubation for 2 h at 37°C, in a humidified 5% CO_2 atmosphere. More than 90% of the adherent cells obtained in this manner were MN as estimated by the non-specific esterase test (α -naphthyl acetate esterase; Sigma Aldrich). MN from each donor were tested prior to ADCI assays and only those without direct inhibitory effect were used in assays.

15

Example 3:

Results

20

The screening of the *Pf* genome using sequence profiles identified several proteins containing putative α -helical coiled coil regions. The regions which are present in the proteome and in the transcriptome databases and have the highest coiled coil scores have been selected for subsequent peptide synthesis. Through proteome and transcriptome data available in the
25 literature (Betts, 2002, Florens et al, 2002, Bozdech et al, 2003) Applicants have assessed if these molecules are present in the erythrocytic stage. The combined analysis/assessment identified 152 coiled-coil regions. Ninety-five coiled-coil segments, 30-70 amino acids long with the highest coiled-coil score, present either in the same protein or in different ones were selected. Most of the selected antigens were recognized at various degrees (5-93%) by a panel
30 of sera from donors living in endemic areas (Table 2).

Thus, 71 new proteins were identified with length varying from 200 to 10,000 amino acids. Affinity chromatography purified antibodies specific for a number of peptides were also obtained from a pool of sera from adult donors living in Tanzania (Table 2). These antibodies are staining infected red blood cells (Fig. 1, Table 3). In addition, one of these proteins

(PF14_0089) was chemically synthesized and tested for recognition in ELISA assays (Table 2). This novel protein is recognized by 78-100% of adult sera from Burkina Faso and Tanzania. Affinity purified antibodies recognize parasite infected red blood cells. In addition, this protein has also been associated with protection in Tanzanian children. These Peptides are also immunogenic in mice and specific sera are positive in IFAT (Table 7). Monoclonal antibodies, which are positive in IFAT, were also isolated.

Table 3

The immunofluorescence antibody test (IFAT) was done on different stages of the parasite life cycle using affinity purified antibodies specific for the most recognized peptides. Results are expressed as weak positive (+/-), positive (+). Inhibition assays on late schizont stages was done to assess the specificity of the staining. Results are expressed as inhibition (+), no inhibition (-) and partial inhibition (+/-) observed.

	IFAT using affinity purified Abs at 10 µg/mL		
SEQ ID ° (proteins)	Early/ring trophozoites	Late schizonts	Inhibition on late schizonts using the peptides at 5 mg/mL
8 (PFB0145c)	+	+	+
9 (PFB0145c)	+	+	-
11 (PFB0145c)	+	+	+/-
12 (PFB0145c)	+	+	+/-
13 (PFB0145c)	+	+	+/-
14 (PFC0245c)	+/-	+/-	+
27 (MAL6P1.37)	+	+	+/-
86 (PF11_0207)	+	+	+/-
102 (PFL1605w)	+	+	+/-
99 (PFL0770w)	+	+	+/-
68 (MAL6P1.147)	+	+	+/-
65 (PFL0250w)	+	+	+/-
134 (PFC0760c)	+	+	+/-
59 (MAL13P1.304)	+	+	-

110 (PF08_0048)	+	+	+/-
78 (PFB0315w)	+	+	+/-
135 (MAL8P1.12)	+	+	-
136 (PF07_0086)	+	+	-
138 (PFC0345w)	+	+	+
146 (PFD0520c)	+	+	+
151 (PFD0970c)	+	+	+/-

ADCI in vitro assay

To wells containing 2×10^5 MN purified as described above, was added 50 μ l of an

5 asynchronous parasite culture at 0.5 % parasitemia and 4 % hematocrit. Wells were then supplemented with test or control Abs and the total volume adjusted to 100 μ l with culture medium. After 48 h and 72 h, 50 μ l of culture medium was added to each well and after 96 h the ADCI assay was stopped and the final parasitemia was determined by light microscopy on Giemsa stained smears by counting $\geq 50,000$ red blood cells. For each antibody (Ab) tested,

10 duplicate wells included the following controls 1) non-specific monocytic inhibition, both MN + parasite, and MN + N-IgG + parasites and 2) direct inhibition by control or test IgG, both N-IgG + parasites, and test Abs + parasites. PIAG and N-IgG were used at a final concentration of 1 mg/ml and as positive and negative controls respectively. Immunopurified test Abs were

15 used at 15 μ g/ml. The specific growth inhibitory index (SGI) which considers the parasite growth inhibition due to the effect of test Abs cooperating with MN was calculated as follows:

$$\text{SGI} = 100 \times [1 - (\% \text{ parasitemia with MN and test Abs} / \% \text{ parasitemia test Abs}) / (\% \text{ parasitemia with MN and N-IgG} / \% \text{ parasitemia N-IgG})].$$

Table 4

ADCI experiments, the results are expressed as the Specific Growth Inhibitory Index (SGI). The positive control (SGI = 100%) used is the IgG fraction purified from a pool of hyperimmune African adults sera which was previously found to confer passive protection when transferred to non-immune patients. α -8, α -9, ... means that the antibody is directed against SEQ ID N8 or N°9 respectively.

5

Affinity Purified Antibodies	SGI (%)
α -8	0
α -9	60
α -11/12	Not tested
α -13	37
α -14	46
α -27	127
α -86	0
α -102	Not tested
α -99	0
α -68	0
α -41	Not tested
α -110	83
α -78	0
α -135	0
α -136	Not tested
α -138	0
α -146	70
α -151	0

Example 4:

5 *Construction of combination antigenic peptides.*

A combination antigenic peptides construct containing different peptide combination (Table) was co-linearly synthesized using the Fmoc chemistry and the Applied Biosystem synthesizer 431 A. To avoid formation of neo-antigenic peptides, individual antigenic peptides were
10 linked via a linker such as for example the immunological silent linker poly-ethylen-glycol (PEG 01-63-0141; NovaBiochem/Merck) as indicated in the table.

Table 5

Peptide	SEQ ID N°	Protein	Sequence
LR162	154	MAL6P1.37(PEG)PFB0145c(PEG)PF14_0089	KKRNVEEELHSLRKNYNIINEEIEEIT(PEG) TISSLSNKIVNYESKIEELEKELKEVK(PEG) NITNINKNIENIKNDMSNLNNMNDNSNQ
LR162A	155	PFA0170c(PEG)MAL6P1.37(PEG) PFB0145c(PEG)PF14_0089	VNNLDSTVNYMNSTGNNINNI(PEG) KKRNVEEELHSLRKNYNIINEEIEEIT(PEG) TISSLSNKIVNYESKIEELEKELKEVK(PEG) NITNINKNIENIKNDMSNLNNMNDNSNQ
LR179	156	MAL13P1.304(PEG) PF08_0048	EKLKKNNEISSLKKELDILNEKMGKCT(PEG) GGLKNSNHNLNNIEMKYNTLNNNMNSINK
LR179A	157	PFB0145c(PEG)MAL13P1.304(PEG) PF08_0048	LDENEDNIKKMKSIDDMEKEIKYR(PEG) EKLKKNNEISSLKKELDILNEKMGKCT(PEG) GGLKNSNHNLNNIEMKYNTLNNNMNSINK
LR181	158	PFD0520c(PEG)PF08_0048(PEG)MAL6P1.37	TKKLNKELSEGKLEKLEKNIKEEETNTLENDIKV(PEG) EKLKKNNEISSLKKELDILNEKMGKCT(PEG) KKRNVEEELHSLRKNYNIINEEIEEIT

15

Table 6

Antibody responses against the peptides of Table 5 using 37 adult sera from Burkina Faso, 42 adult sera from
 5 Tanzania and 39 adult sera from Colombia. Results are expressed as % (value > mean negative control + 3SD),
 ratio (OD exp / mean OD negative control) and not determined (nd).

Peptide	SEQ ID	Burkina Faso sera			Tanzania sera			Colombia sera		
		%	Ratio	Mean OD	%	Ratio	Mean OD	%	Ratio	Mean OD
LR162	154	81	49	0.232	88	21	0.169	54	49	0.293
LR162A	155	97	95	0.477	93	76	0.365	72	51	0.369
LR179	156	68	57	0.323	73	48	0.187	26	15	0.125
LR179A	157	78	57	0.423	83	68	0.317	85	64	0.241
LR181	158	73	68	0.702	68	58	0.391	15	10	0.324

Immunization of CBF1 mice with one antigenic peptide of an antigenic cocktail composition

10 The immunofluorescence antibody test (IFAT) was done on different stages of the parasite life
 cycle using affinity purified antibodies specific for the different peptides and antigenic
 cocktail compositions. Results are expressed as weak positive (+/-), positive (+).

Table 7

Mouse immunization		IFAT		
SEQ ID N°	maximum antibody titres	<i>P. falciparum</i> Sporozoites	Early/ring trophozoites	Mid/late trophozoites
1	218700	-	+/-	-
1	72900	-	+	+
2	100	-	+	+
3	900	-	+	+
35	24300	-	+/-	-
27	24300	-	+	+
38	218700	-	-	+/-
4	72900	-	-	-
34	100	-	-	-
16	100	-	-	-
33	900	-	-	+/-
38	72900	-	-	+
2	100	-	-	-
14	24300	nd	-	-
37	2700	nd	-	-
35	900	nd	-	+
27	24300	nd	-	+
4	2700	nd	-	+/-
34	24300	nd	-	+/-
24	24300	nd	-	+
37	900	nd	-	+/-
133	218700	nd	-	+

Example 5:

Bio-informatics Screening

- 5 The *Plasmodium falciparum* 3D7 genome was used for the bioinformatics analysis (Gardner, 2002). Software named *pftools* and containing programs for sensitive generalized sequence profiles (Bucher et al., 1996) was used to search for the short α -helical coiled coil regions. The coiled coil profiles were constructed using an alignment of amino acid sequences corresponding to the known coiled coil region. Two profiles containing four and five heptad
- 10 repeats were used for the analysis. The coiled coil regions selected by this approach were also tested by the COILS program.
- The selected α -helical coiled coil containing proteins were further tested on their possible surface location and GPI anchoring by using the following programs: identification of potential signal peptides (PSORT, <http://www.psort.org/psortb/>, SignalP, <http://www.cbs.dtu.dk/services/SignalP/>), transmembrane spanning regions (TMPRED http://www.ch.embnet.org/software/TMPRED_form.html and TMHMM <http://www.cbs.dtu.dk/services/TMHMM>), and GPI-anchored proteins http://mendel.imp.univie.ac.at/sat/gpi/gpi_server.html . Furthermore, the presence of the
- 15 identified proteins in the asexual erythrocytic stages was also checked using the published data
- 20 on the transcriptome and proteome of this stage of development of *P. falciparum* (www.PlasmoDB.org).

CLAIMS

1. An antigenic peptide deriving from Plasmodium species comprising at least one coiled
5 coil region, characterized in that said antigenic peptide is selected from the group comprising the amino acid sequences SEQ ID N°1 to 132, SEQ ID N°134 to SEQ ID N°153 and SEQ ID N°159 to SEQ ID N°163, biologically active fragments thereof, molecular chimeras thereof, combinations thereof and/or variants thereof.
- 10 2. The antigenic peptide of claim 1, characterized in that a combination thereof is selected from the group comprising the SEQ ID N° 154, 155, 156, 157 and 158.
3. An antigenic cocktail composition deriving from Plasmodium species comprising at least 2 antigenic peptides according to claims 1 or 2.
- 15 4. The antigenic cocktail composition of claim 3 characterized in that it is selected from the group comprising a combination with
 - i) SEQ ID N° 1, 2 and 3, or
 - ii) SEQ ID N° 16, 33, 38 and 2 or
 - 20 iii) SEQ ID N° 35, 27, 4, 34, 24, and 37.
5. The use of the antigenic peptide of claims 1-2 or of the antigenic cocktail composition of claims 3-4 in the preparation of a vaccine composition for the stimulation of an immune
25 response in a mammal.
6. A vaccine composition useful to stimulate an immune response in a mammal characterized in that it comprises the antigenic peptide of claims 1-2 or an antigenic cocktail composition of claims 3-4.
- 30 7. An antibody characterized in that it recognizes the antigenic peptide of claims 1-2 or the antigenic cocktail composition of claims 3-4.

8. The antibody of claim 7, characterized in that it is selected from the group comprising α -8, α -9, α -11/12, α -13, α -14, α -27, α -86, α -102, α -99, α -68, α -41, α -110, α -78, α -135, α -136, α -138, α -146 and α -151 or mixtures thereof.

5 9. A purified and isolated nucleic acid sequence comprising

- i) a nucleotide sequence encoding an antigenic peptide of claim 1 or 2,
- ii) a nucleic acid sequence complementary to i),
- iii) a degenerated nucleic acid sequence of i) or ii),
- iv) a nucleic acid sequence capable of hybridizing under stringent conditions to i), ii) or iii),
- 10 v) a nucleic acid sequence encoding a truncation or an analog of the antigenic peptide of claim 1 or 2,
- vi) and/or a fragment of i), ii), iii), iv) or v) encoding a biologically active fragment of of said antigenic peptide of claims 1 or 2 .

15 10. An expression vector comprising at least one copy of the purified and isolated nucleic acid sequence of claim 9.

20 11. A host cell comprising a purified and isolated nucleic acid sequence of claim 9 or an expression vector of claim 10.

12. Use of the vaccine composition of claim 6, in the manufacture of a medicament for the treatment and/or prevention of malaria.

25 13. A method of producing an antigenic peptide for the preparation of a vaccine composition against Plasmodium species, characterized in that it comprises the steps of:

- a) identifying the presence of a sequence encoding at least one coiled coil region in the genome of said Plasmodium species,
- b) selecting said sequence encoding at least one coiled coil region,
- 30 c) synthesizing an antigenic peptide corresponding to the sequence containing the at least one coiled coil region selected in step b),

- d) contacting the synthesized antigenic peptide with a serum sample from at least one donor living in Plasmodium species endemic areas; and
- e) determining whether at least one antibody of said serum sample binds to the synthesized antigenic peptide.

5

14. The method of claim 13, characterized in that step a) is performed by a bioinformatic tool.

10 15. A diagnostic tool for determining the presence of the antigenic peptide of claims 1-2 or an antigenic cocktail composition of claims 3-4 in a sample comprising:

- i) contacting the sample with an antibody directed to the antigenic peptide of claims 1-2 or to the antigenic cocktail composition of claim 3-4, and
- ii) determining whether said antibody binds to a component of said sample.

15 16. A diagnostic tool for determining the presence of antibodies to peptide of claims 1-2 or an antigenic cocktail composition of claims 3-4 in a sample comprising:

- i) contacting said sample with the antigenic peptide of claims 1-2 or the antigenic cocktail composition of claim 3-4, and
- 20 ii) determining whether antibodies bind to a component of said antigenic peptide of claims 1-2 or to said antigenic cocktail composition of claims 3-4.

17. A protein characterized in that it comprises at least one antigenic peptide of claim 1.

25 18. The protein of claim 17, characterized in that it is protein PF14_0089 comprising SEQ ID N°38 or protein PFD0520c comprising SEQ ID N° 147.

19. A kit comprising the vaccine composition of claim 6, optionally with reagents and/or instructions for use.